Do We Really Know What is In Our Food? The Connection Between Dietary Mycotoxin Exposure and Pediatric Crohn's Disease

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Do We Really Know What is In Our Food?

The Connection Between Dietary Mycotoxin Exposure and Pediatric Crohn’s Disease

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THESIS

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Dedication

This research is dedicated to my son Dylan, who was diagnosed with moderate-to-severe Crohn’s disease of his entire gastrointestinal tract at age eight. Dylan’s medical course began many years before his actual diagnosis and provided many clues about what foods might be triggering his disease process. Dylan suffered greatly during the first few years of his illness, regardless of our efforts to feed him a wholesome diet.

I kept records of which foods made him sick and which did not throughout those early years. It quickly became apparent that something in the grain supply was bothering him. So much so that he suffered from severe abdominal pain, nausea, vomiting, and fever when he ate them, regardless of his medication regimen. At that time, his growth was also markedly stunted. I initially thought he was gluten intolerant or had Celiac disease; however, Celiac disease was eventually ruled out as a cause. We also discovered that other foods like dairy products affected him similarly.

Then one day, while researching diets used to treat pediatric Crohn’s disease in the University library, the word “Vomitoxin” appeared in a research article and caught my attention. After many hours of research, I discovered that vomitoxin, officially named Deoxynivalenol (DON), belongs to a group of trichothecene fungal mycotoxins that are ubiquitous in the environment and the food supply. Dylan had done a lot of vomiting throughout his illness, and DON had been found to cause abdominal pain, vomiting, diarrhea, headaches, dizziness, fever, and possibly immunological issues in humans. I began digging deeper into the research to see where vomitoxin could be found in the food supply. This thesis represents what was discovered from this initial endeavor.
Acknowledgments

I would like to thank and acknowledge Dr. Pamela DiNapoli for her encouragement and support throughout this research project. Dr. DiNapoli served as my primary advisor and mentor. I am genuinely grateful for the time she spent teaching me about the research process. She encouraged me to follow my passion and combine my background in early childhood development, clinical nutrition, and nursing in this hybrid thesis project. Her encouragement and insights helped to hold me up when I became discouraged. I am genuinely grateful for the time she spent teaching me about the research process and how to do it better.

I would also like to acknowledge my family. Their support of this research helped get me through all the uncertainty, worry, and stress that accompanied being a nurse and the multiple family hardships we experienced during the recent Corona Virus 2019 (COVID-19) pandemic. Without their support and encouragement, this project would not have been possible.

Regarding University laboratory assistance, I would like to thank both Dana Buckley and Amy Michaud for their help in obtaining University approval for this project and for the time they spent educating me on the use of the laboratory. Daryn Daniluk, from Envirologix Corporation, was also instrumental in teaching me proper laboratory techniques for screening flour samples using Envirologix mycotoxin screening equipment.

I would also like to thank the Eta Iota Chapter of Sigma Theta Tau International for a $250.00 grant, which was used to help fund mycotoxin testing laboratory equipment. This equipment was utilized at the University before the pandemic. The pandemic initially waylaid the project. Still, it was able to resume in 2021 by sending samples to Trilogy Analytical Laboratory in Washington, Missouri, for mycotoxin testing instead of using the University Laboratory.
Lastly, I would like to thank Dr. Cathleen Colleran, DNP, RN, and Dr. Gene Harkless, DNSc, APRN, FNP-BC, CNL, FAANP, for their encouragement to complete my work through multiple family hardships. My mother suffered a cerebrovascular accident during the pandemic, for which I was her primary caregiver and nurse. My husband was also diagnosed and treated for head and neck cancer. Without Dr. Colleran and Dr. Harkless’ support and guidance, this project may never have been completed.
Abstract

BACKGROUND: The incidence of pediatric Crohn’s disease (CD) has increased over the past few decades. The etiology of CD has not yet been elucidated. Still, researchers have identified variables associated with the disease process, including genetic predisposition, environmental triggers like a poor-quality diet, air pollution, water pollution, and a dysbiotic microbiome with increased fungal diversity as predisposing factors. Fungal mycotoxin contamination in the food supply from toxicants like Deoxynivalenol (DON), a highly prevalent gastrointestinal irritant, has largely been ignored as a potential factor influencing the fungal dysbiosis and symptoms associated with the disease process. It is hypothesized that global and intermittent exposure to mycotoxins like DON may negatively affect the gastrointestinal health of pediatric CD patients.

OBJECTIVE: The objective of this two-part project was to: 1) Gather evidence of mycotoxin contamination in the food supply, 2) Given the evidence then, to test local food commodities for mycotoxins for the development of a low-mycotoxin diet as a potential treatment modality for pediatric CD.

METHODS: An integrative review of studies measuring global DON prevalence was conducted. With evidence that wheat and corn crops are routinely contaminated with mycotoxins, flours containing these ingredients were directly tested for DON using lateral flow screening technology. Wheat bread and pasta samples were also analyzed and sent to Trilogy laboratory for liquid chromatography, mass spectrometry-mass spectrometry mycotoxin testing.

RESULTS: Results of the integrative review showed that globally, wheat, corn, bakery products, pasta, and mothers’ milk were routinely contaminated with DON. There was also sufficient evidence to suggest that other grain-based crops, soy, coffee, tea, dried spices, nuts, certain seed oils, animal milk, and various water reservoirs are intermittently contaminated. The direct
measurement of foods in a typical child’s diet, such as pasta, bread, and raw ingredients such as wheat- and corn-based flours, also demonstrated routine contamination with DON. Some pasta samples were also contaminated with HT-2 toxin. Contamination rates were significantly higher in 2021 than in 2019, showing the problem may be escalating.

DISCUSSION AND CONCLUSIONS: Universally, due to their increased intake of cereal-based foods relative to their lower body weight it would appear children are at higher risk for exposures to DON than adults. A review of the literature suggests that mycotoxin contamination in the food supply is common. The cumulative effects of multiple mycotoxin exposures by pediatric CD patients may pose serious health risks. Further investigation into the role mycotoxin contamination plays in the disease process, microbial perturbations, and fungal dysbiosis inherent in CD is needed. The information obtained here demonstrates a need to develop a “Low Mycotoxin Diet” for pediatric CD patients to help mitigate the common occurrence of these biohazards.

KEYWORDS: Crohn’s disease, Pediatrics, Deoxynivalenol, Diet, and Mycotoxin
Part 1: Are Pediatric Patients with Crohn’s Disease at Increased Risk for Ill-effects from Dietary Mycotoxin Exposure?

Chapter I

Inflammatory bowel disease (IBD) was referred to as “Fire in the belly” by nurses as early as the 1900s (Brotherton, Taylor, & Keeling, 2013). The prevalence of IBD, which includes both ulcerative colitis (UC) and Crohn’s disease (CD), has increased and is now estimated to affect as much as 3% of the North American population, with approximately 25% of these patients being children (Ashton et al., 2014; Ng et al., 2018). The incidence of pediatric IBD appears to be increasing in areas such as Northern Europe, the United States (US), Canada, and newly developing countries (Ashton et al., 2014; Weimers & Munkholm, 2018). Of the two IBD-related diseases, CD can be more severe, affecting the entire gastrointestinal (GI) tract. CD is characterized by chronic and unpredictable relapsing of intestinal inflammation and injury, leading to weight loss, anemia, malnutrition, reduced bone mass, and growth failure in pediatric patients (Mamula et al., 2002). Children with CD are often significantly smaller and of lower body weight than their age-matched peers (Duricova et al., 2017).

Chapter II: Background

The etiology of CD has not yet been elucidated, but genetic and environmental factors like smoking, air pollution, water pollution (e.g., pesticides and heavy metals), the use of antibiotics, sleep patterns, mode of birth (i.e., cesarean vs. vaginal), stress and a poor-quality diet involving the increased consumption of processed foods with additives, have been implicated in disease development (Dicksved et al., 2008; Hrnčirova, Machova, Trčkova, Krejsek, & Hrnčir, 2019; Lee, D. et al., 2018). Emerging data also implicate microbial dysbiosis with increases in GI fungal diversity and concomitant decreases in bacterial diversity in disease pathogenesis.
Bacterial microbiota changes appear to be characterized by decreases in firmicutes and bacteroidetes communities and increases in pathogenic proteobacteria (e.g., Escherichia coli, shigella, etc.) with concurrent increases in fungal communities like Ascomycota (i.e., candida taxa) and Basidiomycota in children with IBD (El Mouzan et al., 2018; Hoarau et al., 2016; Imhann et al., 2018; Liguori et al., 2016; Mukhopadhyya et al., 2015; Nelson et al., 2020; Tenailleau et al., 2020). Increases in fungal diversity appear to occur at the expense of bacterial diversity, suggesting disease-specific dysbiosis (Hoarau et al., 2016; Sokol et al., 2017). These findings contrast with observations of microbiota in the healthy human gut, which typically displays increased bacterial diversity and decreased fungal diversity (Auchtung et al., 2018; Nash et al., 2017).

Considering these findings, some researchers have waged concerns over the prevalence of mold contaminants like mycotoxins in the human food supply (Maresca & Fantini, 2010; Pestka, 2010a). Mycotoxins are produced by various fungal species (spp.) that frequently invade the food supply and have been largely ignored as potential culprits in the development of the fungal dysbiosis inherent in CD. They are believed to serve as “fitness factors” that help to ensure the survival of various fungal species (Venkatesh & Keller, 2019). They do so by facilitating intra-species communication, limiting communication and reproductive pathways in competing organisms, and at times, by simply killing off competitors, among other functions that assist them in securing an environmental niche (Venkatesh & Keller, 2019).

Many mycotoxins are harmful to humans and are considered a form of foodborne illness that is rarely discussed alongside bacterial forms of food-borne illness (e.g., salmonella, campylobacter, Escherichia coli, etc.) (Keller, Turner, & Bennett, 2005). Mycotoxins in the
environment can enter the human body through inhalation, but many enter through dietary intake. Among the most common fungal contaminants found in the food supply are trichothecene mycotoxins produced by Fusarium fungal spp.

These common field pathogens (e.g., Fusarium graminearum and Fusarium culmorum) routinely infect grains and other crops in the field and storage and produce potent trichothecene mycotoxins like deoxynivalenol (DON), Nivalenol (NIV), Zearalenone (ZEA), HT-2 toxin, and T-2 toxin, among others (Food and Agriculture Organization of the United Nations, World Health Organization, 2016; Maresca & Fantini, 2010; Pestka, 2010a). The most prevalent mycotoxin in the food supply is DON. Trichothecene mycotoxins have been associated with gastroenteritis, emesis, anorexia, and growth retardation in animals and humans and have been found to have deleterious effects on the vulnerable structures of the intestines (Food and Agriculture Organization of the United Nations, World Health Organization, 2016; Keller et al., 2005; Mukhopadhya et al., 2015).

Illnesses caused by mycotoxins abound in the literature including toxic aleukia in the United Soviet Socialist Republic during World War II, red mold disease in Japan, and Balkan nephropathy, among others, and many medically relevant mycotoxins have been identified in foods over the past fifty years (Bennett & Klich, 2003). In healthcare, many food-borne secondary metabolites such as penicillium spp. have received attention for their pharmaceutical merit and potential use as antibiotics. Meanwhile, others identified as food-borne pathogens (e.g., trichothecene mycotoxins) are rarely considered in the pathogenesis of human disease (Bennett & Klich, 2003).
Pathophysiology of DON Exposure

DON exposure appears to initially affect the integrity of the intestinal mucus barrier and possibly, resident microbes. Preliminary evidence shows that mucosal exposure to DON decreases the number of intestinal goblet cells available for mucus production in piglets (Bracarense et al., 2012). Likewise, the same occurs in human muco-secreting cell line HT29-16E at sub-toxic doses, thus potentially decreasing the protective mucus barrier (Bracarense et al., 2012; Pinton et al., 2015; Robert et al., 2017). Chronic exposure to DON also appears to influence microbial defenses and has been found to change gut microbiota (Saint-Cyr, Perrin-Guyomard, Houee, Rolland, & Laurentie, 2013). These effects may be further exacerbated when other trichothecenes and non-trichothecene mycotoxins (e.g., NIV, Patulin, and Ochratoxin A, among others) co-occur with DON, possibly amplifying its toxicity to digestive tract tissues and resident microbiota (Maresca & Fantini, 2010; Pestka, 2008).

After DON penetrates the mucous barrier, it can quickly diffuse through the intestinal mucosa by affecting paracellular tight junctions (TJ), which allows it to become bloodborne as shown in Figure 1 (De Walle et al., 2010; Pestka, 2008; Pestka, 2010b; Pinton et al., 2012). DON appears to affect TJAs by binding to ribosomes, inhibiting protein synthesis in claudin-4 proteins and possibly other claudin and TJ proteins, thereby decreasing adherent junction proteins and increasing intestinal permeability (Bracarense et al., 2012; De Walle et al., 2010). This then appears to cause an increased uptake of DON and higher translocation rates of other luminal antigens such as pathogenic bacterium, viruses, fungi, yeast, food antigens, and other toxic chemicals (Park, Kim, Kim, & Moon, 2015). DON, and possibly foreign antigens, are believed to then trigger an inflammatory response that concurrently activates antigen-presenting cells on T-lymphocytes, resulting in the activation of the adaptive immune system (Akbari et al., 2017;
Al-Sadi, Boivin, & Ma, 2009; Cano et al., 2013; De Walle et al., 2010). This leads to the production of inflammatory cytokines like interleukin (IL)-12 and Th1-mediated tumor necrosis factor-alpha (TNF-α) and interferon-γ (IF-γ) (Al-Sadi et al., 2009; Bracarense et al., 2012; Cano et al., 2013). IF-γ may further affect multiple TJ proteins (e.g., occludins, JAM-A, and claudin-1), increasing TJ permeability (Al-Sadi et al., 2009). In addition, DON has been found to increase the expression of genes involved in the differentiation of Th17 cells like STAT3, IL-17A, IL-1β, and IL-6 and pathogenic Th17 cells such as IL-23A, IL-22, and IL-21 at the expense of regulatory T cells, potentially leading to intestinal inflammation (Cano et al., 2013).

DON has also been found to affect enteric health through two additional mechanisms, transcellular transport, and enterohepatic circulation. By transcellular transport, DON appears to influence nutrient absorption through active uptake via sodium-glucose dependent transport 1 (SGLT1). By affecting SGLT1, DON can impair glucose uptake, leading to an osmotic gradient, diarrhea, and malabsorption of water (Grenier & Applegate, 2013; Maresca, Mahfoud, Garmy, & Fantini, 2002). Facilitated glucose transport is another transcellular pathway that may be influenced by DON uptake via glucose transporter 2 (GLUT2) (Grenier & Applegate, 2013; Maresca et al., 2002). Finally, once DON becomes bloodborne, it is believed to be recycled via enterohepatic circulation. Through this mechanism in animal models, it is secreted with bile and reabsorbed again in the ileum, potentially increasing inflammation at this intestinal site (Grenier & Applegate, 2013; Maresca et al., 2002). Taken together, these processes are believed to increase enteric infection rates in animals and possibly humans (Bracarense et al., 2012; Cano et al., 2013; Grenier & Applegate, 2013; Maresca & Fantini, 2010; Park et al., 2015).
Exposure Limits

Because of its toxicity and widespread prevalence, the Food and Agricultural Organization of the United Nations and the United States (US) Food and Drug Administration (FDA) have issued guidelines for safety limits for animal feed and human food (Food and Drug Administration, 2010; Mishra, Srivastava, Dewangan, Divakar, & Kumar Rath, 2019). Table 1 highlights exposure limits set in Europe and by the US FDA for DON and various other mycotoxins. Acetylated derivatives are not included in these numbers, although 10-20% of DON is believed to exist in acetylated forms that can mask their presence in foods (Food and Drug Administration, 2010; Pinton et al., 2012). In addition to these safety limits, the Joint Food and Agricultural Organization (FAO)/World Health Organization (WHO) and the Expert Committee on Food Additives (JECFA) have set the total Provisional Maximum Tolerable Daily Intake (PMTDI) for DON, and its acetylated forms (i.e., 3-ADON, 15-ADON, DON-3G and DON-15G), at one ug/kg of human body weight. Regrettably, researchers have reported that levels that far exceed the PMTDI may be unavoidable in some populations, and especially in children, due to their high intake of cereal grains relative to their low kilogram body weight (Mishra et al., 2019).

The link between exposure to dietary mycotoxins like DON has not previously been identified as a risk to pediatric CD patients. The primary objective of this integrative review was to search the literature for evidence of widespread mycotoxin contamination in the food supply to support the hypothesis that mycotoxins like DON may be negatively influencing the gastrointestinal health of pediatric CD patients. A secondary objective was to identify where in the food supply pediatric CD patients may be exposed to mycotoxins.
Chapter III: Methods

An integrative review of the literature from 2000 to 2019 was done to identify where in the food supply DON contamination is likely occurring. CINAHL, Medline, PubMed, and the Biological Science Collection databases were searched using the following keywords or phrases both individually and in varying combinations: *deoxynivalenol*, *DON*, *trichothecene*, *mycotoxin*, *food*, *water*, *tea*, *coffee*, *spice*, *nuts*, *milk*, *fusarium*, *infection*, *food contamination*, and *crop contamination*. Occurrences and incidences of DON contamination globally in raw, cooked, baked, processed, and unprocessed foods were sought. Studies showing infection occurrence anywhere from farm to storage to table were included. Only those that looked at contaminated foods for human consumption were included. Surveys and analyses of cereals and other commodities intended for animal feed were excluded. Studies not written in English were also excluded. A total of 118 studies were initially screened. The PRISMA diagram in Figure 2 displays the flow of research selection. Ultimately, one comprehensive systematic review covering global contamination incidences from 2008 to 2018 and 25 other original studies identifying DON, its acetylated metabolites, and other trichothecene mycotoxins were reviewed. Studies selected for review were heterogeneous in terms of testing methods for DON. Studies used various testing methods, such as liquid chromatography combined with mass spectrometry (LC-MS/MS), high-performance liquid chromatography, Elisa testing, and Veratox Fast kits. DON was the most frequently found mycotoxin across all studies. However, DON in its modified forms and other co-contaminant mycotoxins like aflatoxin, ochratoxin A, fumonisin, NIV, T2 toxin, patulin, and a few others were also frequently identified. Crops and foods with greater than 60% contamination incidence were labeled as *routinely* contaminated, and those with lower rates were considered *intermittently* contaminated. Data presented in various studies
were also heterogeneous in terms of units of measure. For ease of clinical interpretation, all data
retrieved from studies were mathematically converted to microgram units. Only studies that
provided descriptive statistics and mean data were included. When multiple samples were
available for a commodity, data were pooled, and the percentage of positive samples was
weighted based on sample size to calculate table data. Studies that provided median and range
data were summarized narratively. Based on evidence of crop contamination with DON, a list of
potentially contaminated foods was developed.

**Chapter IV: Results**

Multiple crops and food commodities were found to be both routinely and intermittently
contaminated with DON either in the field or in storage. Table 2 shows the results of the
synthesized data. These crops and foods included wheat, corn, barley, rye, oats, millet, triticale,
rice, sorghum, grain-derived alcoholic beverages, soy, coffee, tea, dried spices, nuts, and certain
seed oils (Food and Agriculture Organization of the United Nations, World Health Organization,
2016; Garcia-Moraleja, Font, Manes, & Ferrer, 2015; Lee, T. et al., 2011; Mankeviciene,
Suproniene, Brazauskiene, & Gruzdeviene, 2011; Mishra et al., 2019; Raters & Matissek, 2007;
Wu et al., 2014a; Yoshinari et al., 2014). Ready-prepared foods like cereals, pasta/noodles,
bakery products, and even gluten-free foods made from corn and rice were often contaminated
with DON (Mishra et al., 2019; Schollenberger et al., 2005). Virtually any food that could have
been stored for an extended period while awaiting production appeared vulnerable to
contamination.

Studies showed that cow’s milk and possibly milk-based products were potential routes
of DON contamination (Swanson et al., 1986; Winkler et al., 2015). Indirect sources like animal
milk, human milk, and various water reservoirs were also found to be intermittently
contaminated (Dinleyici, Aydemir, Yildirim, Kaya, & Carman, 2018; Kolpin et al., 2014; Ribeiro, Maia, Santos, Tiritan, & Ribeiro, 2016; Schenzel, Schwarzenbach, & Bucheli, 2010; Signorini et al., 2012; Winkler et al., 2015).

Winkler et al. (2015) developed a multi-toxin method for investigating the carryover from animal feed of ZEA, DON, and its various metabolites in cow’s milk. They found that DON and its commonly considered nontoxic metabolite form, de-epoxy-DON (DOM-1), were present in a dose-dependent manner but at low doses (i.e., highest levels were 0.250 ug/L and 0.56 ug/L milk, respectively). These results suggest that when DON is increased in animal feed, it may also be increased in the milk, but in its less toxic form (i.e., DOM-1). Other researchers have found similar results, and that co-contaminant mycotoxins like ZEA and aflatoxin can also find their way into milk, albeit in low doses (Signorini et al., 2012). Aflatoxin M1 was the mycotoxin most frequently found in milk products.

DON has also been found to persist in human milk and infant cereals. A recent study conducted in Turkey on the DON content of breast milk of 90 new mothers found that DON was present in 100% of samples with a median occurrence of 3.9 ug/L (range 0.4-14.99 ug/L) (Dinleyici et al., 2018). Thirty-six percent of breast milk samples contained values over PMTDI recommendations (Dinleyici et al., 2018). Exposure risk to infants was again dependent upon the dietary intake of each mother, and the authors emphasized that DON in breast milk may pose a threat to infant health. In addition, a US study showed that infant cereals were contaminated in 84% of samples at levels ranging from 10-224 ug/kg (n=52, 11 barley, 23 mixed grains, and 18 oats) (Dombrink-Kurtzman, Poling, & Kendra, 2010; Mishra et al., 2019). However, only 1.9% of samples exceeded European limits (Dombrink-Kurtzman et al., 2010; Mishra et al., 2019). In India, 66% of infant cereal samples were DON positive, with levels ranging from 5-228 ug/kg
(n=29) (Mishra et al., 2019). Routine cereal exposures and human milk exposure may increase an infant’s risk of exceeding the PMTDI.

DON has also been found in various water reservoirs in the US and internationally. In a study of 32 streams and three water treatment plants in Indiana, Iowa, and New York, DON was the most prevalent mycotoxin detected (Kolpin et al., 2014). Of 116 samples taken, 77% of stream water samples were contaminated, presumably from crop and animal manure runoff, and 100% of human treatment plant wastewater effluent was contaminated (Kolpin et al., 2014). During snowmelt in Iowa agricultural areas, maximum levels occurred and were as high as 1.66 ug/L. Similar results were found in Switzerland in two earlier studies; however, DON levels were detected in smaller amounts, with the highest recorded level being 0.038 ug/L (Bucheli et al., 2008; Schenzel et al., 2010). In addition, in a study of natural contaminants in the Douro River Estuary in Portugal, DON was again the most prevalent mycotoxin found in the water in concentrations up to 0.374 ug/L. This was thought to result from runoff from crops, livestock effluent, and human wastewater from a local sewage treatment plant. This estuary supplied about 50% of the drinking water for the local urban population (Ribeiro et al., 2016).

Medicinal herbs and spices have also been found to be contaminated with mycotoxins (Do, An, Oh, & Moon, 2015). Researchers in Korea found that multiple mycotoxins commonly contaminate these commodities. Samples from various countries were reviewed, including some from North America. Aflatoxin, ochratoxin A, fumonisins, ZEA, and DON, were the most frequently detected mycotoxins in dried herbal medicines and spices. DON was detected in sage, chamomile, valerian root, senna, dried artichoke, dandelion, rhubarb, boldus, gingko, frangula, lemon verbena, olive leaves, red tea, white tea, spearmint, and star anise. Maximum DON levels varied by herb and ranged from 60 ug/kg to 321.2 ug/kg (Do et al., 2015). Authors attributed
mycotoxin contamination to climate change, poor storage, damage from insects, and infections present at harvest.

Several other food commodities were found to be intermittently contaminated with DON. In a recent study conducted in China, a country that produces as much as 30% of the global rice supply, DON was present in 30.9% of samples (n=236) (Dong et al., 2020). However, after processing by removing the husk and bran to produce white rice, DON content was significantly lowered (e.g., the highest contamination levels reduced from 2,789 ± 301ug/kg to 446 ± 64.8 ug/kg) (Dong et al., 2020). Similarly, in a study conducted in Germany, it was shown that cocoa could become contaminated with DON. However, it was quickly mitigated by removing the outer shell and processing (Raters & Matissek, 2007). In the US, fusarium spp. infections have also been found to cause potato rot with levels in rotted tissue as high as 11,720 ug/L (Delgado, Schwarz, Gillespie, Rivera-Varas, & Secor, 2010). However, infections did not appear to penetrate the entire potato and could be mitigated by removing the rotten tissue within a 3-centimeter margin (Delgado et al., 2010). Whole nuts and seeds were intermittently contaminated with DON, but this appeared to be more dependent on storage conditions than on crop infection (Cunha, Sa, & Fernandes, 2018).

Many foods were found to either show no risk, or limited risk, of contamination with DON. Fresh fruits and vegetables, fresh herbs, honey, maple syrup, table sugar, and salt were not found to be sources of DON. Likewise, dried fruits, fruit juices, and apple-based juices were not found to be sources of DON but were often contaminated with other mycotoxins, such as patulin – another known gastric irritant (Pal, Singh, & Ansari, 2017). Tapioca, arrowroot, and coconut flours were not found to be regularly contaminated with DON. Aged cheeses and butter also did not appear to be significant sources of DON but may be sources of other mycotoxins (e.g.,
Aflatoxin M1) from indirect contamination. Fresh, unprocessed meats and eggs were also not found to be at risk for significant contamination with DON. However, as with dairy products, some researchers believe that mycotoxin exposure from unprocessed meats and eggs may depend on each animal’s diet, but this has not been substantiated by compelling evidence (Alshannaq & Yu, 2017). Data was not found addressing mycotoxin contamination in processed meats.

Chapter V: Discussion

Since the emergence of “Fire in the Belly,” later named CD, this disease has helped shape various nursing specialty areas, including gastroenterology, intensive care, nutrition support, ileostomy care, and wound care. (Brotherton et al., 2013). However, nursing care initiatives for CD were primarily focused on medical treatments and not on the quality of a patient’s usual diet. Throughout this period, nursing practice evolved beyond just implementing guidelines established by the medical community and entered into the scientific discussion to find innovative care solutions for those with CD. Many treatments were developed that nurses championed, like post-surgical ostomy care, bedside delivery of nutrition support (e.g., total parenteral nutrition (TPN) and exclusive enteral nutrition), and medication administration (Brotherton et al., 2013).

Historical documentation of the evolution of CD highlights its increased incidence beginning in the 1900s when industrialized food processors began to dominate the marketplace (Brotherton et al., 2013). New, refined foods (e.g., Wonder bread and Twinkies) made from wheat and other grains became popular during this time. Many North American communities became more dependent upon refined convenience foods to feed their families (Brotherton et al., 2013; Hostess, 2021). As trends toward dependence on refined convenience foods increased, so did the occurrence of CD (Brotherton et al., 2013).
Today, the treatment paradigm has shifted toward re-examining the effects of a poor-quality diet on pediatric CD patients' gastrointestinal health and microbiomes. However, the impact of mycotoxins prevalent in the food supply has remained largely unrecognized by the healthcare community. Notwithstanding, the adverse effects of foodborne mycotoxins on human health have been recognized by the US FDA and the WHO based on already established guidelines to limit their occurrence in human food (Food and Agriculture Organization of the United Nations, World Health Organization, 2016; Food and Drug Administration, 2010). However, the frequency with which mycotoxins appear to be infiltrating the food supply is alarming. DON, in particular, has found its way into a large number of foods placing pediatric patients with CD and possibly those with other GI illnesses (e.g., UC, celiac, and irritable bowel syndrome) at risk for ill effects (Cano et al., 2013; Maresca & Fantini, 2010; Pestka, 2010a).

Low dose, chronic enteric exposures to DON have been found to upregulate cytokines, chemokines, and inflammatory genes leading to anorexia, growth failure, and immunotoxicity in vertebrates due to the resulting “ribotoxic stress” response and inhibition of protein synthesis (Pestka, 2008; Pestka, 2010a). In higher doses, DON has been shown to promote leukocyte apoptosis with concurrent immune suppression, nausea, vomiting, abdominal pain, headache, diarrhea, hemorrhaging, gastroenteritis, dizziness, and fever (Cano et al., 2013; Pestka, Uzarski, & Islam, 2005; Pestka, 2008; Pestka, 2010a). Nurses who care for children should probably be concerned, considering the potential for DON to harm those who repeatedly consume it and its frequent presence in many foods consumed by children.

In recognition of mycotoxins like DON in food commodities as potential public health hazards, the Food and Agriculture Organization of the United Nations and the WHO have reviewed global agricultural and food processing practices related to mycotoxin contamination.
In doing so, they confirmed that fungal spores that cause food contamination are not only found in soil and residues from infected crops but on farming and food processing equipment and within storage structures despite diligent cleaning (Food and Agriculture Organization of the United Nations & World Health Organization, 2016). Factors that affect spore germination involve environmental and storage issues related to humidity and temperature (Alshannaq & Yu, 2017; Food and Agriculture Organization of the United Nations, World Health Organization, 2016).

Humidity and temperature variables that promote mold growth in food can vary from region to region, year to year, season to season, and even storage facility to storage facility. In large storage facilities with limited access to climate control technology, precise temperature and moisture monitoring in stored grains and other commodities may not be possible. Some Fusarium species are remarkably tenacious and require 20% or lower humidity to control their growth which is unlikely to occur in most storage facilities (United States Department of Agriculture, 2006). This problem may be even further intensified when grains or other food commodities are stored for extended periods awaiting production due to food and food processing needs in various regions allowing for a wide variety of tainted foods to make it to the global marketplace unchecked (Food and Agriculture Organization of the United Nations, World Health Organization, 2016). Once present in foods – DON is stable at both high temperatures and under acidic conditions making it difficult to process out (Karlovsky et al., 2016; Mishra et al., 2019; Zhang et al., 2014). Thus, steps involved in food processing (e.g., from farm-to-storage-to-processing-to-table) can result in multiple opportunities for mycotoxin contamination. Because of this, some believe that there may be no way of preventing human mycotoxin exposure from toxins like DON, making them among the biggest threats to the human food
supply, and especially to vulnerable children (Food and Agriculture Organization of the United Nations & World Health Organization, 2016; Keller et al., 2005).

Tracking mycotoxins like DON to ensure food safety and protect vulnerable youth can be expensive and requires specialized equipment. DON and other mycotoxins can be tracked through direct measurement in foods and beverages or by indirect urinary analysis methods to verify their presence (Papageorgiou et al., 2018; Turner, Burley, et al., 2008; Turner, Taylor, White, Cade, & Wild, 2009; Turner et al., 2010). Direct methods (e.g., lateral flow technology, LC-MS/MS, etc.) require testing potentially contaminated food ingredients and discarding contaminated ones before selling them to the public. Indirect methods (e.g., urinalysis) can identify if the patient has consumed a contaminant but cannot identify the food source unless measurements are taken before and after removing a suspected food source. One study in the United Kingdom (UK) demonstrated that by just eliminating wheat intake, urinary measures of DON in humans were decreased from 7.2 ng DON/mg creatinine (95% confidence interval (CI) 4.5-10.5 ng/mg) to 0.6 ng DON/mg creatinine (p<0.001; 95% CI 0.4-0.9 ng/mg) over 48 hours (Turner et al., 2008). Other studies using urinary analysis coupled with food frequency analysis have helped to verify that young children tend to consume higher levels of DON per kilogram of body weight than adults due to the types of foods frequently eaten by them relative to body size (e.g., cereals, pasta, bread, etc.) (Mishra et al., 2019). Some studies involving children have demonstrated intake levels as high as two times the PMTDI limit (Mishra et al., 2019; Papageorgiou et al., 2018).

**Pediatric Diets and Mycotoxins**

There has been an uptick in clinical interest in treating pediatric CD patients with diet for various reasons in recent years. One reason is that young patients frequently remain
malnourished despite a clinician’s best efforts at nutritional remediation. Other reasons are that clinicians have noted a connection between diet and the disease process and that there are significant risks involved with traditional treatments. Various dietary interventions have been explored by looking at nutritional components like sugar, fat, fiber, and fermentable carbohydrates, among other variables (e.g., low-residue diet, the Mediterranean diet, low fermentable oligosaccharide, disaccharide, monosaccharide, and polyol (FODMAP) diet, etc.), but while many have provided short-term symptomatic relief, they have not adequately controlled inflammation or the disease process (Penagini et al., 2016). However, three interventions have shown promise – exclusive enteral nutrition (EEN), the specific carbohydrate diet (SCD), and the Crohn’s disease exclusion diet plus enteral nutrition (EN) (CDED+EN). Each one limits the intake of grains and processed foods, thus limiting dietary mycotoxin exposure to varying degrees.

EEN is typically administered using highly refined, pasteurized formulas that limit exposure to foodborne contaminants in those with active disease while providing a nutritionally complete food source. EEN was one of the first nutritional therapies explored because, beyond total parenteral nutrition, it was the only form of food that these extremely sick patients would tolerate. It was through those initial EEN treatments that caregivers noticed patients getting better. Subsequently, EEN was found to be as effective as steroids at inducing remission based on inflammatory markers and mucosal healing studies without the adverse side effects (Critch et al., 2012; Penagini et al., 2016). Unfortunately, the beneficial effects of EEN appear to be nullified when foods typical to the “Western diet” (e.g., processed foods) are added back (Johnson, Macdonald, Hill, Thomas, & Murphy, 2006). In addition, EEN does not appear to favorably affect the microbiome’s composition. Instead, the use of EEN has demonstrated a
decline in presumptively protective gut bacterial species and metabolites in pediatric patients (Gerasimidis et al., 2014). Until recently, EEN was the only dietary strategy found to control the disease process and, thus, had been the nutritional intervention of choice in the pediatric medical community. However, EEN is expensive, and patients have found it unappealing despite their desire to limit medication use (Suskind et al., 2016). Dietary interventions, on the other hand, have more patient appeal.

The SCD has been one of the most popular dietary interventions used by healthcare providers for patients with CD. The theoretical basis for the diet is to exclude all polysaccharides (i.e., complex carbohydrates) and disaccharides (i.e., sucrose, maltose, isomaltose, and lactose) while allowing only simple sugars in the form of monosaccharides (i.e., glucose, fructose, and galactose) to be included in the diet (Gottschall, 2018). This has been hypothesized to decrease inflammation and increase GI microbial diversity. It excludes all grains, corn, potatoes, soy, starchy vegetables, processed foods, food additives, preservatives, uncultured milk, and sugar; and includes honey, fruits and vegetables, nuts, nut-derived flours, beef, fish, poultry, fermented yogurt, dry-curd cottage cheese (lactose removed), and hard cheeses. Because of its recent popularity, the SCD has been one of the most studied diets to date and is nutritionally adequate to meet growth needs in children (Braly et al., 2017). From data obtained in this review, the SCD diet limits any significant exposures to DON by eliminating all grains, processed foods, and most animal milks unless microbiologically cultured in some way. Preliminary evidence suggests it may have a marked effect on inflammatory markers and effectively induce remission in those with mild-to-moderate CD. It also appears to improve intestinal microbial diversity, but only in those who follow the diet strictly. Inflammation appears to return once the diet is liberalized.
PEDIATRIC CROHN’S DISEASE AND DIETARY MYCOTOXINS

(Burgis, Nguyen, Park, & Cox, 2016; Penagini et al., 2016; Suskind et al., 2018). Considering its effects on the microbiome, more research into the diet’s impact on the mycobiome is needed.

The CDED+EN is another diet gaining momentum in popularity with healthcare providers and has shown promise for inducing remission in those with mild-to-moderate CD. The CDED+EN is more tolerable than EEN alone. It can be described as a whole food diet coupled with EN designed to limit exposure to dietary components that have been found to have adverse effects on the intestinal barrier and microbiome (Levine et al., 2019a; Sigall Boneh et al., 2017). The diet limits or eliminates exposure to animal fats, certain types, and cuts of meats, gluten, maltodextrin, xanthan gum, emulsifiers, sulfites, and specific monosaccharides (Levine et al., 2019b). In the initial phases of the diet, only fresh, unprocessed meats, freshly prepared fruits and vegetables, fresh herbs, fresh potato, white rice and rice noodles, honey, monounsaturated sources of oil (e.g., olive and canola), and herbal teas, among other freshly prepared items are included. Not included in the diet are wheat, corn flour, all grains except for white rice, all dairy products except for isolated dairy proteins provided by enteral formulas, breakfast cereals, bread, and baked goods, gluten-free products, soy, potato flour, dried fruit, processed meats, margarine, seed oils and packaged snacks like popcorn, nuts, chips, chocolate, cakes, cookies and gum (Sigall-Boneh et al., 2014). Utilizing only fresh foods with minimal processing appears to be the foundation of this diet aside from the EN used for nutritional supplementation. Evidence suggests that the diet is not only nutritionally complete but that it may have a marked effect on inflammatory markers and may be effective for inducing remission. Based on data available in tables 1 and 2, this dietary intervention appears to limit exposure to multiple foodborne mycotoxins in addition to DON (e.g., ochratoxin A, aflatoxin, and patulin). Moreover, intestinal microbial diversity has shown improvement in “responders” to this diet, which researchers
believe may have a protective effect on participants once the diet is liberalized (Levine et al., 2019a; Sigall Boneh et al., 2017). This claim has yet to be substantiated through long-term clinical evidence. Future research into the diet’s effects on the mycobiome is also needed here.

**Microbial Shifts Toward Fungal Dysbiosis in CD**

Gut-dwelling microorganisms in humans appear to be involved in many physiological functions that involve detoxification processes, metabolism of nutrients, production of short-chain fatty acids, and induction of the host immune response (Chin et al., 2020). The human gut hosts a myriad of organisms involved in these processes, including bacteria, fungi, Archie, and viruses (Chin et al., 2020). Until now, gut-dwelling bacteria that comprise the “microbiome” have received the most attention due to their relative abundance in the intestines. However, recent technological advancements have allowed for identifying the fungal species that coexist with bacteria in this extra-intestinal environment and have been dubbed the “mycobiome.”

The oral cavity provides a direct entry route for microorganisms like fungi into the gastrointestinal tract and is responsible for enumerable exposures throughout life (Suhr & Hallen-Adams, 2015). Infants are borne with extraordinarily little fungal diversity, although candida spp. colonization appears to occur during vaginal birth from the mother’s microbiota. Fungal inhabitants are thought to steadily increase as children age due to various environmental exposures like diet. There are an estimated 100 trillion microbes in the human gut by adulthood, and these microorganisms outnumber human cells ten to one (Suhr & Hallen-Adams, 2015). Fungi typically only make up about one percent of this number in comparison to bacterial inhabitants in healthy individuals (Suhr & Hallen-Adams, 2015).

Imbalances in these extra-intestinal microbes have been found to affect the host’s health. Conversely, host environmental changes (e.g., a diet high in processed foods, toxic exposures,
medical treatments, etc.) can adversely affect microbial communities (Lewis et al., 2017). Shifts in microbial communities in pediatric CD (e.g., decreases in gut bacterial diversity and increases in fungal diversity) appear to be specific to the disease process and are believed to precede the onset of disease in children (Chehoud et al., 2015; El Mouzan et al., 2018; Mukhopadhya et al., 2015; Sokol et al., 2017).

Increases in fungal diversity, in turn, are believed to affect detoxification processes, genetic expression, and host immunity in CD (Nelson et al., 2020). Nelson et al. (2020) found that a predominance of Ascomycota fungi (e.g., Candida spp.) in CD patients appeared to be a significant contributing factor in fungal dysbiosis and inflammation. Hoarau et al. (2016) also found that candida (c.) spp., and particularly c. tropicalis, were the predominant fungal species in CD patients. In addition, they observed significant intra-kingdom correlations between c. tropicalis and five other known fungal genera: Fusarium spp., haematonectria, nectria, thanatephorus, and trichosporon. All these fungal species have been identified as foodborne pathogens in agricultural communities. Fusarium spp., haematonectria, and nectria are all Ascomycota fungi and common plant pathogens that have been found to infect wheat, corn, and potato, among other crops (Lenc, Kwasna, Jeske, & Joriczyk, 2016). Thanatephorus and trichosporon are also foodborne pathogens, with the former being a common field pathogen and the latter being a form of yeast found in contaminated dairy products – both are Basidiomycota fungi (Grosch, Scherwiniski, Lottmann, & Berg, 2006; Kasahara, Ishikawa, Sato, Shimakawa, & Watanabe, 2014). Many of these fungal species have demonstrated the ability to form mycotoxins like DON (e.g., Fusarium spp.) outside of the human gut, but little is known about their ability to do so within the human intestine. Little is also known about whether these organisms can colonize the intestines of those with CD or if they were simply “passing through.”
Fusarium fungal species do not appear to colonize the mycobiota of healthy subjects (Auchtung et al., 2018).

Hoarau et al. (2016) also identified relationships between proteobacteria like Escherichia coli (e. coli) and Serratia marcescens (s. marcescens), and c. tropicalis in those with CD. Alliances between candida and proteobacteria appeared to result in the formation of biofilms that were significantly thicker and more protective of their microbial niche collectively than could be provided by each organism separately (Hoarau et al., 2016). Interkingdom relationships between fungi and proteobacteria were not observed in healthy control subjects indicating that these collaborations may also play a role in disease pathogenesis (Hoarau et al., 2016). The relationship between other fungal species identified in the study and proteobacteria was not elucidated.

Hoarau et al. (2016) also did not elucidate communication systems between species. Still, secondary metabolites (i.e., mycotoxins) and chemical mediators have facilitated this in nature (Bennett & Klich, 2003). Fungi often utilize mycotoxins and other localized mediators for bacterial-fungal and fungal-fungal communication (Venkatesh & Keller, 2019). This communication is believed to facilitate microbial assembly processes between species that aid in the formation of protective mixed biofilms in varied niches (Venkatesh & Keller, 2019). Another function is that they provide protection against other microbes and have been shown to wage “antibiotic warfare” that limits microbial competition for resources – helping them secure an environmental niche (McCormick, 2013; Venkatesh & Keller, 2019). Additionally, mycotoxins are involved in disrupting quorum sensing and signaling between bacteria. Quorum signaling allows for cell-cell communication allowing bacteria to share information about cell population density and adjust gene expression accordingly. Blocking this pathway may give
fungi an advantage over certain bacteria (Venkatesh & Keller, 2019). As technology improves, the influence of fungal secondary metabolites on disease-specific microbial shifts and interkingdom communication may be better elucidated.

**Mycotoxins and Microbes**

Historically, fungal species and their associated metabolites and chemical mediators have been studied for medicinal purposes and have been used to create pharmacological agents like antibiotics. Many of the fungal-borne secondary metabolites used to develop antibiotics are similar to mycotoxins, except they primarily target bacteria. On the other hand, mycotoxins are toxic to animals, humans, and certain bacterial species (Bennett & Klich, 2003). Patulin is an example of a mycotoxin commonly found in apple juice in low concentrations; that was once studied for its potential use as an antibiotic due to its effects on bacteria but was found to cause nausea and gastritis in humans and was thus, relegated to the category of “mycotoxin” (McCormick, 2013). Antibiotics and mycotoxins are potent weapons against various microbes – some potentially affecting the microbial diversity of the human gut. Chronic exposure to DON, like Patulin, has been found to affect gut microbiota (Saint-Cyr et al., 2013). Hundreds of mycotoxins have been identified since the 1960s, with the most common route of exposure being from dietary contact (Bennett & Klich, 2003).

Bacterial detoxification of fungal-borne mycotoxins appears to be an essential function of some bacterial species. Certain bacteria, like fungi, have also been found to produce secondary metabolites that inactivate and, at times, completely degrade mycotoxins (McCormick, 2013). There are many examples of bacterial detoxification of mycotoxins in nature. Mycotoxin-degrading bacteria have been found in soil contaminated with polycyclic hydrocarbons, possibly due to their structural similarities (McCormick, 2013). Gram-positive lactic acid bacteria have
been shown to bind various mycotoxins (e.g., zearalenone and aflatoxin), rendering them harmless. Specific yeast, including saccharomyces, rhodotorula, and cryptococcus spp., have been shown to detoxify ochratoxin A – a common contaminant in cereals, coffee, and chocolate (Alshannaq & Yu, 2017; McCormick, 2013). Fermentation with saccharomyces cerevisiae in fruit juice to produce vinegar removes patulin (McCormick, 2013). Many microbes have been found to detoxify trichothece mycotoxins like DON, such as lactobacillus rhamnosus, bacillus (b.) subtilis, b. coagulans, b. velezensis and clostridium spp. WJ06, among others (Czaczyk, Trojanowska, & Mueller, 2002; Garcia et al., 2018; Li, Wang, Huang, Chen, & Wang, 2017; McCormick, 2013; Rabbee et al., 2019; Zhao et al., 2014). Animals (e.g., ruminant species) have also demonstrated an ability to detoxify DON to its less toxic form de-epoxy DOM (DOM-1) (Winkler et al., 2015). However, microbes in their digesta are believed to help facilitate this for the animal (Grenier & Applegate, 2013; McCormick, 2013). Only humans who live with and handle livestock have demonstrated the ability to detoxify DON to DOM-1 (Turner et al., 2010).

**Nursing Implications**

It is unlikely that pediatric CD patients with decreased microbial defenses can detoxify contaminants like DON or other mycotoxins in food. From the evidence, it appears that repeated exposure to mycotoxins in foods frequently eaten by children may pose health risks (Keller et al., 2005; Mishra et al., 2019). It is unclear if the common occurrence of mycotoxin contamination in food and drink plays a major or minor role in the microbial perturbations and GI distress experienced by pediatric CD patients. However, it is clear that limiting mycotoxin exposure by avoiding processed foods and large amounts of grains may help mitigate CD symptoms. Consequently, it is imperative for nurses who care for these patients to understand the evidence either supporting or against specific dietary interventions.
Preliminary evidence suggests that both the SCD and the CDED+EN may positively impact inflammation and mucosal healing in pediatric CD patients. The CDED+EN appears to be the “cleanest” diet in that it limits mycotoxin exposure to a greater extent than the SCD – at least in its initial phases. Nurses, and other healthcare staff, can safely recommend both diets in conjunction with medical treatments. Each is at least as nutritionally sound as the average American diet – if not more so (Braly et al., 2017; Levine et al., 2019b). Because malnutrition is common in pediatric CD, supplementation with EN to make up for shortfalls may also be prudent.

Nurses should be aware that the biggest concern involved with treating this disease with specialized diets is adherence – especially as children get older. Frequent, intensive support is necessary. Nutritional intervention studies involving the SCD and CDED+EN indicate that both lost patients due to their inability to follow the diets (Levine et al., 2019b; Suskind et al., 2018). Moreover, in most studies, patients appeared to have difficulty maintaining the diets strictly beyond three months, which may undermine their therapeutic usefulness. Nurses can play a pivotal role in case management by being aware of environmental, personal, and self-care deficit barriers that interfere with adapting to successful health-related behaviors.

Nurses are uniquely positioned to provide such guidance and support to patients and families to improve adherence to a treatment plan. A plan that includes food preparation is essential. Nurses should educate patients and families on not only what to eat but on alternatives to favorite foods when patients are having difficulty complying with prescribed treatments. Care using an interdisciplinary team approach that includes a physician, a nurse, and a dietitian is needed to promote optimal wellness and disease control across the spectrum of care.
Limitations and Future Implications

This integrated review is limited by the heterogeneity of the methods used in various studies to detect mycotoxin exposure in food commodities. Methods of detection have steadily improved over the past decade. To add value to this body of knowledge and better quantify pediatric risk, researchers should attempt to quantify the presence of DON and other mycotoxins in food commodities using a more standardized and precise approach (e.g., LC-MS/MS) (Kresse, Drinda, Romanotto, & Speer, 2019). In addition, more research is needed to explore contamination rates in foods frequently eaten by children like cereals, pasta, pizza, dairy products, and even drinking water to assess the magnitude of mycotoxin prevalence better and to provide data for the development of a mitigation plan aimed at protecting vulnerable youth. Future research using validated urinary markers may also help to clarify the extent to which young patients are being exposed to mycotoxins and are subsequently exceeding the PMTDI limit.

To better understand the relationship between exposure to these toxins and fungal dysbiosis, well-controlled clinical trials focusing on lowering dietary mycotoxins with simultaneous analysis of its effects on the human mycobiome should be designed. Identifying the role of fungal secondary metabolites like DON, and other chemical mediators, involved in inter- and intra-microbial communication systems in those with CD may lead to new treatment options to control the inflammatory disease process.

Chapter VI: Conclusion

CD is an incurable inflammatory disease that can be devastating and debilitating to children. Eliminating harmful contaminants from the diets of these children has potential protective benefits from the health hazards that DON and possibly other mycotoxins pose
Preliminary evidence suggests global food mycotoxin contamination rates are significant and could represent considerable safety risks for those with a diminished microbiome and already compromised GI function. The cumulative effects of multiple exposures to DON and perhaps other mycotoxins found in pediatric diets require the clinician to consider the harmful effects they may have on the microbiomes of pediatric patients. Informed guidance regarding alternative treatments that limit food-borne contaminants like DON and their impact on the disease process may give pediatric patients their best chance to maintain remission and good health throughout life.
Part 2: Pediatric Crohn’s Disease and Dietary Mycotoxin Exposure: A Pilot Study

Assessing Dietary Mycotoxin Content in Select Wheat and Corn Products

Chapter I

Crohn’s disease (CD) is a severe, incurable form of inflammatory bowel disease (IBD) that is characterized by transmural inflammation involving any portion of the gastrointestinal tract from the oral cavity to the perianal area (Peppercorn & Kane, 2022). CD’s clinical course is often more rapid and aggressive, with more extensive intestinal involvement in children than adults (Duricova et al., 2017). The incidence of pediatric IBD has been increasing since the turn of this century in areas such as Northern Europe, the United States (US), Canada, and newly developing countries (Ashton et al., 2014; Weimers & Munkholm, 2018). In Wessex, England alone, the incidence of pediatric CD has increased by as much as 50% over the past decade (Ashton et al., 2014).

Managing the care of these patients has historically required intensive nursing care, the administration of intravenous and oral medications, surgical procedures, wound care, and ostomy care, among other treatments (Ford et al., 2011; Steenholdt et al., 2015). The estimated annual cost of these treatments can range anywhere from $30,000 to $133,692 per patient (Fondell, Mosha, Frank, Brangi, & Hyams, 2020). Furthermore, most treatments are potentially unpleasant for pediatric patients and have psychological ramifications, such as diminished quality of life and depression (Chrobak-Bien, Gawor, Paplaczyk, Malecka-Panas, & Gasiorowska, 2017; Claar et al., 2017; Nicholas et al., 2007; Ojeda & Cofre, 2018). Pediatric CD patients face a lifetime of such medical treatments if other means of treatment are not established.

Because of the devastating physical, financial and psychological implications of this disease process and the association between “Western-style” diets and disease prevalence, diet
has recently been explored as an alternative treatment option. Nurses have assumed a primary role in the care of pediatric CD patients making it essential to understand the evidence surrounding various treatment modalities, including diet.

**Chapter II: Background**

Dietary intake of mycotoxins for some populations – and especially children – is substantial (Mishra et al., 2019). The most prevalent mycotoxin in the food supply is deoxynivalenol (DON). Dietary DON exposure can increase intestinal permeability, potentially promoting increased uptake and translocation of other luminal antigens such as pathogenic bacterium, viruses, fungi, yeast, food antigens, and other toxic chemicals (Bracarense et al., 2012; De Walle et al., 2010; Park et al., 2015). Through this mechanism, DON is believed to increase enteric infection rates in animals and possibly humans (Bracarense et al., 2012; Cano et al., 2013; Grenier & Applegate, 2013; Maresca & Fantini, 2010; Park et al., 2015). Enteric infections often plague young CD patients. Consequently, such exposures may represent considerable safety risks to pediatric patients with already-compromised GI function.

**Evidence of DON in the Food Supply**

Preliminary evidence suggests contamination rates for DON in the global food and water supply are significant (Gonya, 2021). Wheat and corn have been the most routinely contaminated crops, with mean levels, at times, exceeding US Federal Food and Drug Administration (FDA) and European pediatric exposure limit guidelines (Gonya, 2021). Products frequently consumed by children, such as baked goods (e.g., bread, pasta, cakes, etc.), have also been found to be routinely contaminated (Mishra et al., 2019). Multiple, frequent, daily exposures may represent chronic, cumulative pediatric exposures and pose considerable safety risks for pediatric CD patients.
The objective of this pilot study was to assess the prevalence of DON in a small number of foods and raw ingredients frequently eaten by children to further evaluate the need for the development of a low-mycotoxin diet as a potential treatment modality for pediatric CD. DON was chosen as a primary mycotoxin target for this study because of its known prevalence in the food supply, its adverse effects on the human gastrointestinal tract, and its ability to persist throughout the manufacturing process (Karlovsky et al., 2016). To date, dietary exposure to mycotoxins like DON has not been directly addressed by therapeutic diets used to treat this vulnerable population.

Chapter III: Methods

Two of the most commonly contaminated grain-based flours, wheat and corn, and wheat-based bread and pasta, were selected for direct testing for DON. The Envirologix QuickScan II, Lateral Flow Reader ACC-331, and DON Flex test, strips AQ-304-BG were used to screen flours. High-performance liquid chromatography coupled with mass spectrometry-mass spectrometry (LC-MS-MS) was used for testing wheat-based bread and pasta samples. All samples were collected from different manufacturers from three different stores in Southern New Hampshire (NH) for pilot testing. Flours were purchased and tested during two separate growing seasons from September 30, 2019, to October 30, 2019, and again from December 1, 2021, to December 13, 2021. Bread and pasta samples were purchased and tested between February 1, 2021, and June 30, 2021. All samples were obtained from the front of each supermarket display shelf. Packaging was inspected for product integrity before purchase to ensure there were no rips or tears and that all samples were fresh and within coded expiration dates.

Wheat and corn flours were tested following standardized Envirologix QuickScan II screening equipment procedures. Procedures included ensuring that greater than 95 percent of
the collection sample could pass through a number 20-mesh (i.e., 850 um) sieve. None of the flour samples required grinding to reduce their particle size. Then, a 20-gram sample was measured out and combined with 100 milliliters (mL) of reverse osmosis filtered water in a clean, sealable, disposable container. The container was vigorously shaken for 30 seconds. The mixture was then poured through a bleached coffee filter into a beaker for extract sampling. Next, in a separate test tube, 100 microliters (uL) of DB6 dilution buffer solution were combined with 100 uL of extract using a fresh pipette and then mixed with the pipette tip. The test tube was placed in an incubator to acclimate to 22°C Celsius (C) for two minutes. After this, a QuickTox DON Flex test strip was placed in the solution for an additional two minutes. The bottom pad of the strip was then cut per manufacturer instructions, and the strip was placed in the QuickScan carrier to read the results. Three tests were done for each item, and results were quantified using descriptive statistics.

To test for DON in bread and pasta, samples were sent via US Postal Service Priority Mail to Trilogy Analytical Laboratory (Washington, MO) to be analyzed for both group A and B trichothecene mycotoxins using LC-MS-MS procedures. Using LC-MS-MS laboratory methods allowed for more complex matrices like bread and pasta to be analyzed accurately. To date, lateral flow screening technology has not been validated for foods with complex matrices containing multiple ingredients. Methods used by Trilogy laboratory to detect mycotoxins in food commodities are described elsewhere with only a few differences (Kresse et al., 2019). Trilogy’s methods used an extraction of 84:16 acetonitrile/water, whereas Kresse et al. (2019) used an extraction of 80:20 acetonitrile/water. In addition, Trilogy lab used a purification column before injection on the instrument to aid in better chromatography allowing for the detection of
22 mycotoxins, including DON, instead of just 16. These methods were otherwise not locally available during the Coronavirus disease 2019 (COVID-19) pandemic.

Chapter IV: Results

Flour and food samples obtained from three Southern NH stores were sourced from various regions in the US and abroad (e.g., Vermont, Maine, Maryland, Oregon, Texas, Italy, Columbia, etc.). Ingredients for bread and pasta may also have been sourced from multiple locations domestically and abroad, but this information was not disclosed on the packaging. As a result, while samples were selected from a small catchment area, most products tested were sourced from various locations outside of NH. DON contamination rates in all tested commodities greater than or equal to 50% were labeled as “routine” exposures, and rates less than 50% were labeled as “intermittent.” Table 3 shows mycotoxin contamination rates for all products tested. All products tested were found to be routinely contaminated.

Wheat and Corn Flours

In 2019, wheat flours were found to be contaminated with DON in 60% of samples (mean = 0.117 parts per million (ppm), SD = 0.10 ppm), and corn flours were found to be contaminated in 50% of samples (mean = 0.342 ppm, SD = 0.189 ppm) using lateral flow methods. The contamination rate of wheat flour samples markedly increased from 2019 to 2021, with 100% of wheat flour samples testing positive for DON in 2021 (mean = 0.248 ppm, SD = 0.100) and with higher mean toxin levels. Corn flour samples also showed an increase in the incidence of DON contamination from 2019 to 2021, with 100% of samples testing positive for DON in 2021, but mean levels remained stable (mean = 0.317 ppm, SD = 0.285 ppm). However, the range of levels detected in corn was wider in 2021 than in 2019. The highest levels recorded in corn samples in 2021 were 0.77 ppm and 0.56 ppm, which exceeded European safety limits.
for all consumers. All told, European safety limits were exceeded for infants and children in 66% of wheat flour and 50% of corn flour samples in 2021.

**Wheat-based Bread and Pasta Samples**

DON was detected in 100% of wheat bread samples using LC-MS-MS methods, with higher mean levels found in bread samples than in wheat flour samples. Fifty percent of wheat-based pasta samples were also contaminated with DON. Mean levels for pasta products were also found to be higher than mean levels found in wheat flours, with the range of contamination varying more widely than could be shown by mean and standard deviation data. DON levels in contaminated pasta samples ranged from 0.4 ppm to 1.0 ppm, with 1.0 ppm being at the upper limit of allowable for US citizens. One pasta sample exceeded European safety limits for adults, children, and infants. Two pasta samples were contaminated with an additional trichothecene mycotoxin – HT-2 toxin – which, unlike water-soluble DON, is fat-soluble but with similar gastrointestinal effects. One pasta sample was contaminated with both DON and HT-2 Toxin. All told, wheat-based pasta and bread samples were contaminated with at least one mycotoxin 75%-100% of the time, respectively.

**Chapter V: Discussion**

Numerous diets and nutritional strategies have been evaluated over the past several years for their ability to induce remission in CD (Penagini et al., 2016). Yet, little attention has been given to the secondary metabolites produced by various fungi found in the human diet other than for their pharmaceutical merit. Indeed, many mold-borne secondary metabolites have been used to create medications like penicillin, cyclosporin, statins, and numerous other lifesaving drugs (Keller et al., 2005). However, while potentially useful pharmaceutically, Trichothecenes may
cause considerable GI illness after repeated exposures to tainted food (Keller et al., 2005; Mishra et al., 2019).

Historically, the most difficult crops to control for DON contamination have been wheat, barley, rye, and corn (Mishra et al., 2019). Consistent with the literature, findings in this pilot study reflect routine contamination from both wheat and corn sources. All told, 60% of wheat flour samples and 50% of corn flour samples were DON positive in 2019. These samples were not found to exceed safe levels set by the US FDA. However, one wheat flour sample (e.g., DON level 0.233 ppm) and two corn flour samples (e.g., DON levels 0.5 ppm and 0.39 ppm) exceeded European guidelines for infants and children in 2019. Wheat flours sampled in 2021 were contaminated 100% of the time and with higher levels. Likewise, corn flours were contaminated 100% of the time in 2021 and with a wider range of levels. None of the 2021 samples exceeded US FDA guidelines, but European guidelines were routinely exceeded. This was not surprising, considering European guidelines for infants and children are stricter and cover a wider range of foods than US guidelines. However, multiple, cumulative, daily exposures to contaminated foods could quickly lead to children exceeding US FDA limits. It is not clear why DON contamination rates were higher in 2021, but it is possible that the COVID-19 pandemic influenced this occurrence in some way (e.g., extended storage times).

Wheat-based bread samples were also contaminated with DON 100% of the time, and wheat-based pasta samples were contaminated with at least one mycotoxin 75% of the time. One pasta sample was particularly concerning with two mycotoxins, DON and HT-2 toxin. Finished wheat products were also found to have consistently higher levels of DON than wheat flours, which may have been due to climate conditions and crop fungal infections in the areas from which ingredients were sourced and time ingredients spent in storage.
The detection of HT-2 toxin in two pasta samples was unexpected. HT-2 toxin has been shown to cause toxicity by inhibiting DNA and RNA function and, ultimately, protein synthesis (Pettersson, 2011). Like DON, it can cause nausea, vomiting, and other unpleasant GI side effects (Bennett & Klich, 2003). HT-2 toxin is often considered together with T-2 toxin, which can be rapidly converted to HT-2 after ingestion. Both appear interchangeable and have similar effects on mammals (Pettersson, 2011). T-2 toxin and HT-2 toxin are believed to be more toxic than DON, and their cytotoxic effects can cause immunosuppression and decreased resistance to infectious microbes (Bennett & Klich, 2003). T-2 toxin is believed to be responsible for an outbreak of alimentary toxic aleukia (ATA) in the United Soviet Socialist Republic (USSR) in the Orenburg district during World War II. Symptoms of ATA toxicity include vomiting, diarrhea, leukopenia, intestinal hemorrhage, and at times, shock and death (Yagen & Joffe, 1976). Citizens who became ill from ATA had eaten “overwintered” grain harvested after the snow melted that was colonized with fusarium sporotrichioides and fusarium poae (Bennett & Klich, 2003). There is currently no US regulatory guidance available for T-2 or HT-2 toxin exposure levels in foods. In addition to DON and HT-2 toxin, fusarium fungi can produce a variety of trichothecene mycotoxins (e.g., NIV, ZEA, Fusarenon, Diacetoxyscirpenol, etc.), many of which can have harmful effects on humans (Bennett & Klich, 2003).

The Fusarium Pandemic

Fusarium fungi were originally found to thrive in the cool, wet climates of Northern Europe, North America, Canada, Russia, and China, among other cooler climates. These areas of the world are where the symptoms of Crohn’s disease were first recognized. One of the earliest probable cases of Crohn’s Disease was that of King Alfred the Great in Wessex, England (849-900 AD) (Craig, 1991). It was said that his disease began after a 24-hour grand feast in
celebration of his marriage to Ealhswith, his Mercian bride, when he was approximately 19 years old. Before this, however, he had suffered from piles (i.e., hemorrhoids), which some have speculated were peri-anal lesions (Craig, 1991). These lesions were said to have disappeared miraculously just before his severe attack of pain during his wedding feast. From that day onward until his death, he suffered from intermittent diarrhea, constipation, rectal bleeding, splenic pain, and “internal tenderness.” The Journal of the Royal Society of Medicine determined that based on a differential diagnosis of church records, Crohn’s disease was the likely culprit for Alfred’s relapsing and remitting gastrointestinal problems, which finally took his life at age 50 (Craig, 1991).

King Alfred would not have eaten the highly processed foods complete with additives available in today’s markets, but he would have afforded some frequent luxuries like bread and cakes. Based on the local crops grown and foods eaten in this area of the world during Alfred’s reign, these foods would have been made from wheat, barley, oats, or rye (Blair, Keynes, & Scragg, 2014). Furthermore, many of the grains used to make foods would have been stored in cool, dark, somewhat damp places – all conditions conducive to mold growth. He may have also consumed potentially contaminated well water, animal milk, or mead and beer made from barley. Wessex has recently experienced alarming increases in pediatric CD, which may have some genetic underpinnings that date back to Alfred’s time. However, repeated exposures to environmental contaminants like fusarium-borne mycotoxins – all indigenous to this area of the world – may not be helping matters.

Since just before the turn of this century, fusarium species (spp.) crop infections have spread to newly developing countries and become a global problem for various agricultural reasons. Countries like Africa now regularly grow wheat, corn, and other grains that potentially
harbor fusarium infections (Mishra et al., 2019). Because of this, DON has been estimated to have affected the growth of nearly 160 million children younger than five years old globally, causing the World Health Organization (WHO) to recognize its presence in crops as a significant threat to human health (Food and Agriculture Organization of the United Nations, World Health Organization, 2016). The pandemic-like spread of these fungal crop infections has also coincidently overlapped with increases in IBD incidence in newly developing countries (Ng et al., 2018). Further, these same infected crops (e.g., wheat and corn, among others) are heavily used by industry to produce the “Western-style” diet commonly implicated in CD development.

Controlling the spread of Fusarium infections in grain-based crops has been a complex problem that governing bodies have been unable to rectify. Many reasons for this include climate change, inappropriate crop rotation between infected plant species (e.g., wheat and corn) instead of rotating crops with infection-resistant species, reduced tilling practices, and insect damage, among other variables (Food and Agriculture Organization of the United Nations & World Health Organization, 2016). Furthermore, the modern-day use of pesticides and fungicides has done little to control their occurrence (Food and Agriculture Organization of the United Nations & World Health Organization, 2016). Some fungicides, like those from the strobilurin group, may even encourage fusarium growth by stimulating mycotoxin production through a chemical-pathogen interaction that is not well understood (Sydenham & De Villiers, August 2016). The strobilurin fungicides have been found to increase DON levels by six to eighteen percent in harvested grain after treatment (Sydenham & De Villiers, August 2016). Once present in foods, mycotoxins like DON are resistant to high heat and chemical food processing and, consequently, can remain in the finished products like baking flours, bread, and pasta (Karlovsky et al., 2016; Magan et al., 2010). Because of all these and perhaps other variables, some believe that
preventing and mitigating mycotoxin infiltration into processed foods might not be possible (Food and Agriculture Organization of the United Nations, World Health Organization, 2016). Unfortunately, this problem is only expected to increase due to climate change (Parikka, Hakala, & Tiilikala, 2012).

**Pediatric Exposure Risks**

Agricultural communities have long been aware that crop fungal infections and their associated mycotoxins can negatively impact the growth and development of livestock and increase the occurrence of enteric infections (Grenier & Applegate, 2013). Agricultural communities have adapted to these biohazards by providing mycotoxin binders and targeted probiotics in contaminated feed. At present, there is no such mitigation strategy for the human food supply other than to limit exposure. Furthermore, the Joint Food and Agricultural Organization (FAO)/WHO and the Expert Committee on Food Additives (JECFA) guidelines for safe intake limits (e.g., the Provisional Maximum Tolerable Daily Intake (PMTDI) for DON is one ug/kg of body weight) appears to be routinely exceeded by children, possibly due to their increased intake of cereal grains relative to their smaller body size (Mishra et al., 2019; Papageorgiou et al., 2018). Urine studies have demonstrated that some European children consume as much as two times the PMTDI for DON – despite stringent exposure guidelines. Thus, current attempts to limit exposure do not appear to be adequately protecting children.

While the samples collected in this pilot study reflect low-dose exposures to DON, the potential for multiple, cumulative, repeated exposures in youth is what is most worrisome. Both low and high-dose DON exposures can stimulate an immune response that adversely alters the function of intestinal TJs and increases intestinal permeability – a pathophysiological occurrence that has otherwise been referred to as a “leaky gut.” This, in turn, could result in increased uptake
of other enteric pathogens found in food such as Mycobacterium Avium subspecies Paratuberculosis (MAP), Listeria, Campylobacter, and Escherichia coli, among others (Maresca & Fantini, 2010; McNees, Markesich, Zayyani, & Graham, 2015; Pestka, 2010a). MAP is a pathogen that has recently sparked great research interest due to its ability to cause a similar disease to human CD in dairy cows, meat cattle, and other livestock – Johne’s Disease (McNees et al., 2015). MAP infections are apparently common in dairy herds and some beef herds and can survive pasteurization (McNees et al., 2015). The chemical effects of mycotoxins like DON on enteric cells and TJs, combined with food-borne pathogens like MAP, may place vulnerable youth at even higher risk for ill effects. These effects may then be further exacerbated when other mycotoxins like HT-2 toxin co-occur with DON, possibly amplifying its toxicity to digestive tract tissues (Maresca & Fantini, 2010; Pestka, 2008).

**Nursing and Dietary Considerations**

Based on data collected here and in other studies, it is not a matter of “if” young patients will be exposed to harmful mycotoxins like DON but of “how much” they will be exposed to. Until now, the frequent appearance of DON in various food commodities – and even water – has not been addressed by therapeutic diets designed to treat children with CD. In fact, while pediatric CD patients often suffer from an increased incidence of enteric infections, food safety from any pathogen is rarely discussed within the context of dietary interventions aimed at this group. Instead, the focus has been historically on dietary macronutrient composition (e.g., fat, carbohydrates, simple sugars, etc.), dietary fiber, the removal of additives from processed foods, and on improving diet quality by increasing the intake of fresh whole foods, among other nutrition-based improvements (Penagini et al., 2016). No doubt, a poor-quality diet consisting of overly processed foods high in sugar and fat can negatively impact human health and are worthy
targets in nutrition interventions. However, toxicants like DON lurking within these foods may also negatively impact human health and need to be dealt with.

Florence Nightingale once observed that lack of clean water, poor nutrition, and sanitation were crucial issues contributing to disease in humans. Throughout her life, she remained steadfast, recording data related to improving sanitation and hygiene at the hospital in which she worked. Procedural developments that included washing the linens and towels, purchasing necessary kitchen supplies, and emphasizing handwashing with soap and water, which were not otherwise widely practiced, were put into place. Regardless of these measures, through 1855, the mortality rate at the hospital in which she worked continued to rise (Oerther & Oerther, 2020). A Sanitary Commission eventually found that the hospital was built on a sewer, the water supply was contaminated, and it was helping to increase the spread of disease. These scenarios abound in the literature and continue today even with improved technology for detecting food and waterborne biohazards like mycotoxins. Like Nightingale’s contaminated water in 1855, contaminants like DON remain an unrecognized threat that is potentially harmful to vulnerable youth.

Some dietary interventions available to clinicians used to treat pediatric CD inadvertently limit mycotoxin exposure. This is done by either eliminating table food altogether or by limiting processed foods, grains, and some animal milks that potentially contain these contaminants. These interventions have shown promise for decreasing gastrointestinal inflammation. Several examples of such dietary interventions are exclusive enteral nutrition (EEN), the Specific Carbohydrate diet (SCD), and the Crohn’s disease exclusion diet plus enteral nutrition (CDED+EN) (Levine et al., 2019b; Suskind et al., 2018). All three dietary interventions limit
grain intake, and each limits mycotoxin exposure to varying degrees – especially from grains that harbor fusarium-produced trichothecene mycotoxins.

All three dietary interventions have been difficult for patients to comply with for various social and cultural reasons. Patients have found EEN to be unappealing despite their desire to limit medication use (Suskind et al., 2016). In addition, while dietary interventions have had more patient appeal than EEN, they have been notoriously difficult for young patients to comply with long-term because they can be very restrictive. Nutritional intervention studies involving the SCD and CDED+EN indicate that both lost patients due to their inability to follow the diets strictly beyond three months (Levine et al., 2019b; Penagini et al., 2016; Suskind et al., 2016; Suskind et al., 2018). Most interventional diets, including these, require sick children to eat differently from their peers, leading to feelings of isolation that many may not be amenable to. Other pediatric patient populations with dietary constraints, such as those with Celiac disease, have demonstrated that very restrictive diets that do not consider food preferences are not only challenging to comply with but can negatively affect social and emotional well-being (Mager et al., 2018; Wolf et al., 2018). Thus, strict dietary interventions that do not consider pediatric preferences may undermine their quality of life and, ultimately, a diet’s therapeutic usefulness.

Florence Nightingale was aware of the social issues surrounding food intake and devoted an entire chapter in her “Notes on Nursing” to diet (Oerther & Oerther, 2020). In this chapter, she highlighted the need to address food and water safety and the importance of attending to the personal food preferences of the patient, the family, and the community to return a patient to good health (Oerther & Oerther, 2020). Considering that diet therapy is being recommended more frequently now in the treatment of pediatric CD, nurses should be cognizant of these concerns when assessing young patients, especially since current dietary therapies may not be
adapted well enough to US pediatric preferences for long-term use. Developing a “Low Mycotoxin” diet that both attends to pediatric preferences and removes toxicants like DON from young diets may be a viable way to address this problem.

**Limitations, Strengths, and Future Research Implications**

The data collected in this pilot study are limited by the small sample size and the use of only one small region in Southern NH for obtaining samples. Unfortunately, limited funding limited the number of samples that could be tested. Likewise, the variety of foods commonly eaten by children that could affordably be tested was also limited.

Strengths include that those flours purchased in local stores were sourced from multiple regions globally and that they were evaluated during two different years, which helped to corroborate previous research findings demonstrating that DON contamination in some wheat and corn products may be chronic. Additionally, using a third-party lab, Trilogy laboratory, that utilized LC-MS-MS to analyze bread and pasta samples allowed for more accurate results and allowed for the detection of multiple mycotoxins in each sample. Because of this, the use of LC-MS-MS, or equally accurate methods, is recommended for future studies evaluating food-borne mycotoxins rather than the single mycotoxin screening technology used in this study for testing flour samples.

A future large-scale study evaluating the mycotoxin content of multiple foods typically eaten by children in various US regions is recommended to better assess exposure risk to vulnerable youth. Direct measurement of products commonly consumed by children (e.g., crackers, bread, cereals, pasta, pizza, dairy products, and even tap water) may help to better quantify the risk to pediatric CD patients. Methods such as urinalysis can also be used to assess DON exposure in youth. Previous studies assessing DON’s presence in foods through urinalysis
in children and adults have demonstrated it may be a valid way to track exposure (Papageorgiou et al., 2018; Turner et al., 2008; Turner, Rothwell et al., 2008; Turner et al., 2009; Turner et al., 2010). A closer look at how repeated exposures to mycotoxins like DON affect intestinal micro- and mycobiota and the fungal dysbiosis inherent in CD is also needed.

Lastly, considering that this and other studies have found that DON contamination rates in grain-based foods may be chronic, direct testing of alternative ingredients (e.g., tapioca, coconut flours, etc.) that could be used to replace grain-based ingredients in the development of a “Low Mycotoxin Diet” is proposed. A “Low Mycotoxin Diet” that provides foods that children are amenable to may help to mitigate for the common occurrence of these biohazards in pediatric diets.

Chapter VI: Conclusion

CD is an incurable inflammatory disease that can be devastating and debilitating to children. Considering the potentially harmful effects of DON on gut health, the routine dietary intake of contaminated foods by children with CD may pose serious health risks. The development of a “Low Mycotoxin Diet” that can be adapted to patient preferences represents a new treatment option that could not only impact the disease process but ultimately help to extinguish the “fire in the belly.”
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doi:10.1128/mBio.01250-16 [doi]


doi:10.1177/1757913920908888 [doi]


doi:10.1039/c7tx00138j [doi]


Sigall-Boneh, R., Pfeffer-Gik, T., Segal, I., Zangen, T., Boaz, M., & Levine, A. (2014). Partial enteral nutrition with a crohn's disease exclusion diet is effective for induction of remission in children and young adults with crohn's disease. *Inflammatory Bowel Diseases, 20*(8), 1353-1360. doi:10.1097/MIB.0000000000000110


Table 1. Exposure limits set in Europe and the US FDA for Mycotoxins by Crop.

<table>
<thead>
<tr>
<th>Mycotoxin and effects on humans</th>
<th>Fungal Species</th>
<th>Food Commodity</th>
<th>US FDA (ug/kg)</th>
<th>Europe (ug/kg)</th>
</tr>
</thead>
</table>
| **Aflatoxin B1, B2, G1, G2**  
*Hepatotoxic and hepatocarcinogen* | Aspergillus flavus  
Aspergillus parasiticus | Maize (corn), wheat, rice, peanut, sorghum, pistachio, almond, ground nuts, tree nuts, figs, cottonseed, spices | 20 for total | 2-12 for B1  
4-15 for total |
| **Aflatoxin M1**  
*Hepatotoxic and hepatocarcinogen* | Metabolite of Aflatoxin B1 | Milk, milk products | 0.5 in milk | 0.5 in milk  
0.25 in infant formula and infant milk |
| **Ochratoxin A**  
*Kidney tumors and nephropathy* | Aspergillus ochraceus  
Penicillium verrucosum  
Aspergillus carbonarius | Cereals, dried vine fruit, wine, grapes, coffee, cocoa, cheese | Not set | 2-10  
0.5 in products for infants |
| **Fumonisins B1, B2, and B3**  
*Disrupts sphingolipid metabolism in cell membranes and may be a causal factor in esophageal cancer.* | Fusarium verticilliodes  
Fusarium proliferatum | Maize and maize products, sorghum, asparagus | 2000-4000 | 200-1000 |
| **Zearalenone**  
*An estrogenic hormone disruptor in some animals and possibly humans.* | Fusarium graminearum  
Fusarium culmorum | Cereals, cereal products, maize, wheat, barley | Not set | 20-100 |
| **Deoxynivalenol and Nivalenol**  
*Targets actively dividing cells such as those lining the GI tract, skin, lymphoid and erythroid cells. Esophageal cancer. Categorized as a “ribotoxin” and protein synthesis inhibitor.* | Fusarium graminearum  
Fusarium culmorum | Cereals, cereal products, maize, wheat, rye, buckwheat, oats, millet, triticale, rice, sorghum, alcoholic beverages made from cereal grains. | 1000 in finished wheat products like flour, bran and germ, 500 in cereal-based foods for infants and children | 200-500 in processed grains  
200 in products for infants |
| **Patulin**  
*Immune suppression, carcinogenesis, gastrointestinal inflammation, ulcers, bleeding, etc.* | Penicillium Expansum  
Aspergillus Byssochlamys | Apples (rotten), apple juice and concentrate (can also occur in other fruits, dried fruits and juices) | 50 | 10-50 |

Sources for the table: (Alshannaq & Yu, 2017; Food and Drug Administration, 2010; Mishra et al., 2019; Pal et al., 2017; Park et al., 2015; Turner et al., 2008).
Table 2. Deoxynivalenol (DON) contamination in the global food supply.

<table>
<thead>
<tr>
<th>Crop/food Tested &amp; Reference(s)</th>
<th>Countries Represented</th>
<th>Total Number of Studies (N)</th>
<th>Total Sample Size (n)</th>
<th>Percentage Positive Samples (%)</th>
<th>Mean and Standard Deviation in ug/kg or ug/L (liquids)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wheat</strong> (Mishra et al., 2019; Yoshinari et al., 2014)</td>
<td>Argentina, Brazil, Canada, China, India, Iran, Israel, Italy, Netherlands, Serbia, Romania, Uruguay, Japan, Sweden, Slovakia, Finland, Hungary, Switzerland, Poland, Nigeria, Spain</td>
<td>33</td>
<td>4520</td>
<td>89%</td>
<td>878 $\pm$ 679 ug/kg</td>
</tr>
<tr>
<td><strong>Maize (Corn)</strong> (Mishra et al., 2019; Yoshinari et al., 2014)</td>
<td>Hungary, Portugal, Serbia, Japan, South Africa</td>
<td>7</td>
<td>615</td>
<td>79%</td>
<td>1041 $\pm$ 1525 ug/kg</td>
</tr>
<tr>
<td><strong>Barley</strong> (Mishra et al., 2019; Yoshinari et al., 2014)</td>
<td>Argentina, Brazil, Romania, Japan, Tunisia</td>
<td>6</td>
<td>386</td>
<td>48%</td>
<td>2,629 $\pm$ 1684 ug/kg</td>
</tr>
<tr>
<td><strong>Bakery Products</strong> (Mishra et al., 2019)</td>
<td>Brazil, Hungary, Serbia,</td>
<td>3</td>
<td>398</td>
<td>80%</td>
<td>375 $\pm$ 306 ug/kg</td>
</tr>
<tr>
<td><strong>Noodles &amp; Pasta</strong> (Mishra et al., 2019)</td>
<td>Italy, Germany</td>
<td>2</td>
<td>67</td>
<td>89%</td>
<td>275 $\pm$ 159 ug/kg</td>
</tr>
<tr>
<td><strong>Oats</strong> (Mishra et al., 2019)</td>
<td>Finland, Sweden, United Kingdom</td>
<td>3</td>
<td>427</td>
<td>50%</td>
<td>1359 $\pm$ 1882 ug/kg</td>
</tr>
<tr>
<td><strong>Rice</strong> (Yoshinari et al., 2014)</td>
<td>Japan</td>
<td>1</td>
<td>60</td>
<td>40%</td>
<td>18 $\pm$ NA ug/kg</td>
</tr>
<tr>
<td><strong>Soy</strong> (Schollenberger et al., 2007)</td>
<td>Germany</td>
<td>1</td>
<td>45</td>
<td>13%</td>
<td>67.2 $\pm$ 96.2 ug/kg</td>
</tr>
<tr>
<td><strong>Azuki beans</strong> (Yoshinari et al., 2014)</td>
<td>Japan</td>
<td>1</td>
<td>40</td>
<td>38%</td>
<td>12 $\pm$ NA ug/kg</td>
</tr>
<tr>
<td><strong>Nuts</strong> (Cunha et al., 2018)</td>
<td>Portugal</td>
<td>1</td>
<td>14</td>
<td>36%</td>
<td>2.85 $\pm$ NA ug/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Almonds)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3 (Cashews)</td>
<td>100%</td>
<td>135.8 $\pm$ NA ug/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7 (Hazelnuts)</td>
<td>86%</td>
<td>336.5 $\pm$ NA ug/kg</td>
</tr>
<tr>
<td><strong>Cow’s Milk</strong> (Signorini et al., 2012)</td>
<td>Argentina</td>
<td>1</td>
<td>704</td>
<td>40%</td>
<td>0.388 $\pm$ NA ug/L</td>
</tr>
<tr>
<td><strong>Vegetarian Milks</strong> (Hamed, Arroyo-Manzanares, Garcia-Campana, &amp; Gamiz-Gracia, 2017)</td>
<td>Spain</td>
<td>1</td>
<td>8 oat milk</td>
<td>38%</td>
<td>22.7 $\pm$ 3.99 ug/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8 soybean milk</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 rice milk</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 nut milk</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td><strong>Coffee</strong> (Garcia-Moraleja et al., 2015)</td>
<td>Spain</td>
<td>1</td>
<td>169</td>
<td>43%</td>
<td>67 $\pm$ NA ug/Kg</td>
</tr>
<tr>
<td><strong>Tea</strong> (Reinholds, Bogdanova, Pugajeva, &amp; Bartkevics, 2019)</td>
<td>China (Pu-Erh tea)</td>
<td>1</td>
<td>70</td>
<td>90%</td>
<td>1,952.2 $\pm$ 646 ug/kg</td>
</tr>
<tr>
<td></td>
<td>Latvia (herbal tea)</td>
<td>1</td>
<td>60</td>
<td>45%</td>
<td>1,198 $\pm$ NA ug/L</td>
</tr>
<tr>
<td></td>
<td>Lebanon (Kaak tea)</td>
<td>1</td>
<td>20</td>
<td>50%</td>
<td>70 $\pm$ NA ug/kg</td>
</tr>
</tbody>
</table>
Mean data from various studies were combined to calculate the average incidences of contamination in samples. Contamination rates were found to vary from month-to-month, product-to-product and region-to-region depending on weather conditions, storage and food processing circumstances, among other reasons.

<table>
<thead>
<tr>
<th>Seed oils (Rapeseed and Linseed)</th>
<th>Lithuania</th>
<th>1</th>
<th>Rapeseed</th>
<th>62</th>
<th>75%</th>
<th>12.46 ± 7.81 ug/Kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Linseed</td>
<td>32</td>
<td>100%</td>
<td></td>
<td></td>
<td>25.06 ± 12.13 ug/Kg</td>
</tr>
</tbody>
</table>

Table 3. Mycotoxin Testing Results
<table>
<thead>
<tr>
<th>Matrices, sample size, and year</th>
<th>Percentage of contaminated lateral flow test samples</th>
<th>* LC-MS-MS results for group A trichothecenes</th>
<th>** LC-MS-MS results for group B trichothecenes</th>
<th>*** Mean value in ppm with standard deviation (SD) Group A trichothecene results are in ppb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat flour, n=6, 2019</td>
<td>60% PV</td>
<td>PV</td>
<td>PV</td>
<td>0.117 ppm (117 mcg), SD = 0.103 ppm (103 mcg)</td>
</tr>
<tr>
<td>Corn flour, n=6, 2019</td>
<td>50% PV</td>
<td>PV</td>
<td>PV</td>
<td>0.342 ppm (342 mcg), SD = 0.189 ppm (189 mcg)</td>
</tr>
<tr>
<td>Wheat flour, n=6, 2021</td>
<td>100% PV</td>
<td>PV</td>
<td>PV</td>
<td>0.248 ppm (248 mcg), SD = 0.10 ppm (100 mcg)</td>
</tr>
<tr>
<td>Corn flour, n=6, 2021</td>
<td>100% PV</td>
<td>PV</td>
<td>PV</td>
<td>0.317 ppm (317 mcg), SD = 0.286 ppm (286 mcg)</td>
</tr>
<tr>
<td>Whole Wheat Bread, n=3, 2021</td>
<td>NA ND</td>
<td>100%</td>
<td>0.3 ppm (300 mcg), SD = 0.1 ppm (100 mcg)</td>
<td></td>
</tr>
<tr>
<td>Wheat pasta for DON, n=4, 2021</td>
<td>NA ND</td>
<td>50%</td>
<td>0.35 ppm (350 mcg), SD = 0.47 ppm (470 mcg)</td>
<td></td>
</tr>
<tr>
<td>Wheat pasta for HT-2 Toxin, n=4, 2021</td>
<td>NA ND</td>
<td>9.02 ppb, SD = 10.61 ppb</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NA = Not Applicable; ND = None Detected; ppm = parts per million; ppb = parts per billion
PV = LC-MS-MS previously validated with lateral flow testing with good reliability.

*Group A Trichothecenes are Diacetoxyscirpenol, HT-2 Toxin, T-2 Toxin, and Neosolaniol;
**Group B Trichothecenes are Deoxynivalenol, 15-Acetyl Deoxynivalenol, 3-Acetyl Deoxynivalenol, Nivalenol, and Fusarenon X.

***US FDA DON exposure guidelines: DON cannot be sold for human consumption in amounts greater than 1.0 ppm in finished wheat products like flour, bran, and germ (Food and Drug Administration, 2010). European allowable limits for children and adults are 0.2 ppm - 0.5 ppm in processed grains, respectively, and 0.2 ppm in products intended for infants (Mishra et al., 2019).

Figure 1. Potential effects of DON exposure on the enterocytes.
Figure 2. Research Flow Diagram.

- **Mucus Layer**: Deoxyribonucleic acid (DNA) exposure may decrease the number of goblet cells, potentially decreasing the protective mucus barrier.

- **Paracellular Route**: Tight junction disruption and increased permeability leading to:
  - Increased translocation of mycotoxins, pathogenic bacteria, viruses, food antigens, etc.
  - Stimulation of immune defenses and lymphocytes.

- **Transcellular Route**: Impairment of glucose and other nutrient uptake.

- **Lymphocytes**: Lymphocytes respond to DON and other foreign antigens by producing inflammatory cytokines.

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Figure 2: Research Flow Diagram

Identification

- Records identified through database searching: CINAHL, Medline, PubMed, and the Biological Science Collection (n = 75)
- Additional records identified through study references (n = 48)

Screening

- Records after duplicates removed (n = 118)
- Records screened (n = 118)

Records excluded:
1. Animal feed studies
2. Studies conducted prior to the year 2000
3. Studies not written in English (n = 65)

Eligibility

- Full-text articles assessed for eligibility (n = 53)

Full-text articles excluded:
1. DON not included in mycotoxins studied
2. Data not available
3. Food commodities not typically consumed by children (e.g., alcoholic beverages) (n = 27)

Included

- Studies included in the review (n = 26)